Stability of Green Tea Catechins in Commercial Tea Leaves during Storage for 6 Months

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[Correction added after online publication Feb. 23, 2009: M. changed to Mendel]

ABSTRACT: To help meet the needs of consumers, producers of dietary tea products, and researchers for information on health-promoting tea ingredients, we determined by HPLC 7 catechins [(−)-epigallocatechin (EGC), (−)-catechin (C), (+)-epicatechin (EC), (−)-epigallocatechin 3-gallate (EGCG), (−)-gallocatechin 3-gallate (GCG), (−)-epicatechin 3-gallate (ECG), and (−)-catechin 3-gallate (CG)] in samples of 8 commercial green tea leaves of unknown history sold as tea bags in the United States, Korea, and Japan. The samples were stored at 20 °C and sampled at 1 wk and 1, 2, 4, and 6 mo. The following ranges in the initial values (0 controls) were observed (in mg/g tea leaves): EGC and C, 0 to trace amounts; EC, 1.9 to 21.1; EGCG, 13.3 to 113.0; GCG, 0.2 to 1.6; ECG, 5.7 to 50.5; CG 0.5 to 3.7; total catechins 36.5 to 169.7. Statistical analysis of the results and plots of changes in individual and total catechin levels as a function of storage time indicate a progressive decrease in the content in the total levels, most of which is due to losses in the most abundant catechins, EGCG and ECG. Possible mechanisms of degradations of catechins during storage and the possible significance of the results to consumers of tea are discussed.

Keywords: degradation, HPLC, storage, tea catechins, tea leaves

Introduction

Tea leaves produce organic compounds that may be involved in the defense of the plants against invading phytopathogens and are reported to have beneficial effects when consumed as part of the human diet (Ho and others 2009). These secondary metabolites include polyphenolic tea catechins (Figure 1). In the course of previous studies designed to determine the composition of commercial tea leaves and the inhibitory activities of tea ingredients against human cancer cells and foodborne pathogenic bacteria (Friedman and others 2006a, 2007), we noted that the catechin content of the same commercial tea leaves decreased when analyzed at a later date. These observations suggested the possibility that catechins in commercial tea leaves may not be stable during long-term storage in the solid state. Because tea is not susceptible to spoilage, it can be stored for extended periods of time. However, degradation of catechins during storage of commercial tea leaves in warehouses, restaurants, stores, and the home could adversely impact tea's potential health benefits.

Because earlier reports on factors that influence the degradation of tea catechins in liquid media, including tea infusions, and in solid tea extracts are relevant to the theme of the present study, we briefly summarize these studies in chronological order: (1) Tea catechins were stable in acid solutions at pH values < 4, exhibited intermediate stability in the pH range of 4 to 8, were highly unstable at pH > 8, and were stable in boiling water for up to 7 h (Zhu and others 1997); (2) The stability of catechins in commercial tea drinks during storage was affected by pH and added ingredients. The following conditions minimized degradation of tea catechin in tea drinks: controlled temperature (4 °C); pH 5.5; an atmosphere low in oxygen; storage in the dark; the use of purified, metal-free water; and the presence of additional stabilizing catechins (Chen and others 1998, 2001; Su and others 2003; Labbé and others 2008); (3) EGCG added to animal drinking water underwent auto-oxidation and epimerization, which was prevented by the antioxidative enzyme superoxide dismutase (SOD) and a nitrogen atmosphere (Sang and others 2005); (4) Thermally induced simultaneous degradation and epimerization of EGCG in water followed 1st-order kinetics (Wang and others 2008); (5) About 17% of EGCG and EGC and 9% of ECG and EC added to doughs were lost during baking (Wang and Zhou 2004). Whether the presence of catechin antioxidants during bread baking prevents acrylamide formation in the bread crust (Friedman and Levin 2008; Hedegaard and others 2008; Mizukami and others 2008) merits study; (6) Individual and total catechins in a spray-dried green tea extracts containing (to simulate commercial instant tea products) combinations of sugar, citric acid, ascorbic acid, or no additives were stable during storage for 3 mo at a relative humidity below 43% (Ortiz and others 2008); (7) EGCG, EGC, and ECG were significantly degraded during *in vitro* gastric and small intestinal digestion (Neilson and others 2007). The loss of catechins was accompanied by the formation of homo- and hetero-catechin autooxidation dimers (Tanaka and others 2002); and (8) Previously, we found that day-old teas exhibited significantly lower antimicrobial activities against the foodborne pathogen *Bacillus cereus* than did freshly brewed teas (Friedman and others 2006a).

The cited and related studies suggest that catechins in liquid and solid environments may undergo degradation, oxidation, epimerization, and polymerization reactions forming new food ingredients. The mechanisms of these transformations and their significance for the human diet remain largely unknown. The main objective of the present study was to assess whether degradation of catechins takes place in processed and packaged teas after their purchase at local markets and during storage under home-like conditions.

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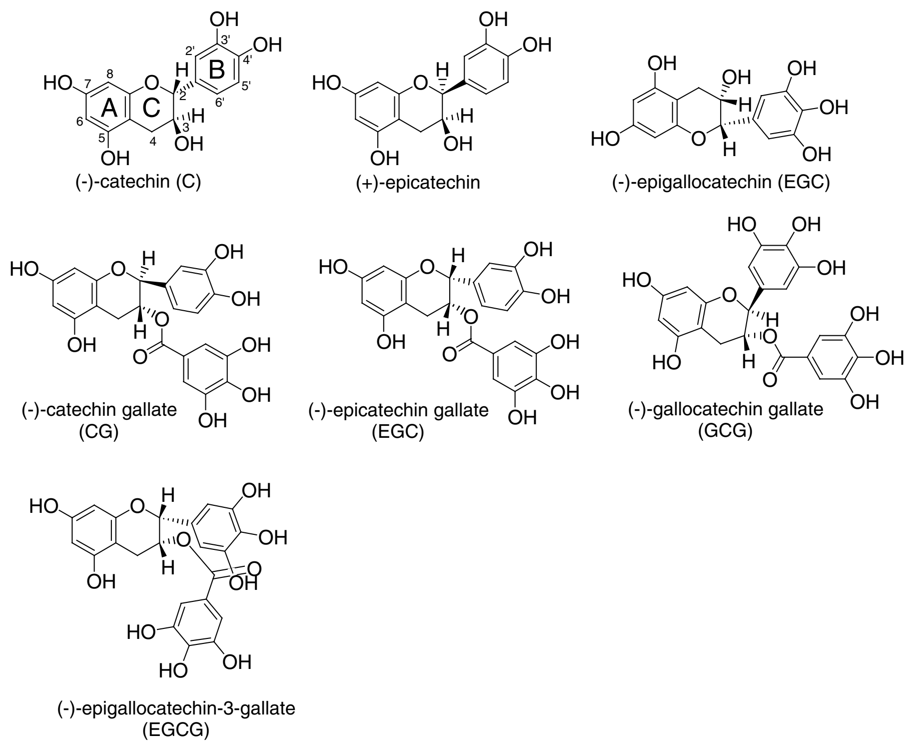

Figure 1 — Structures of green tea catechins evaluated in the present study. The indicated abbreviations are used in the text.

Materials and Methods

Materials

The following catechin standards were obtained from Sigma/Aldrich (St. Louis, Mo., U.S.A.): (−)-epigallocatechin (EGC), (−)-catechin (C), (+)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), and (−)-catechin gallate (CG). Table 1 lists the 8 green teas purchased as tea bags from commercial sources in the United States, Japan, and Korea. HPLC solvents were filtered through a 0.45-μM membrane (Millipore, Bedford, Mass., U.S.A.) and degassed in an ultrasonic bath before use.

Table 1 — Eight green teas evaluated in the present storage study.

Tea nr	Tea brand	Source
1	Organic Darjeeling Green	Brand A, U.S.A.
2	Organic Green Blend Green	Brand A, U.S.A.
3	Susi Bar Green	Brand A, U.S.A.
4	Moroccan Mint Green	Brand A, U.S.A.
5	Dragonwell Green	Brand A, U.S.A.
6	Kukicha Green	Brand B, Japan
7	Sencha Green	Brand C, Japan
8	Uzen Green	Brand D, Korea

Storage and extraction conditions of tea leaves

Tea bags were stored in the original containers in the dark at room temperature (20 °C) for 5 time periods: 1 wk and 1, 2, 4, or 6 mo. For analysis, tea leaves were finely ground into a powder with a mortar and pestle or a homogenizer. The powders (0.5 to 0.6 g) were mixed with 100 mL of water previously brought to the boiling point (average temperature of the water was approximately 90 °C). The sample was then stirred slowly with a magnetic stirrer for 5 min. The cooled sample was filtered through a 0.45 μM Millipore nylon filter before analysis.

HPLC analysis of tea catechins and alkaloids

We had previously developed and validated an HPLC method for the extraction and analysis of catechins, theaflavins, and the methyl-xanthine alkaloids caffeine, theobromine, and theophylline present in commercial tea leaves (Friedman and others 2005, 2006b). The method was used to separate in a single run, 7 catechins, 4 theaflavins, and 3 alkaloids. This method with minor modifications was used in the present study to quantify the distribution of 7 catechins present in 8 commercial green teas.

HPLC was carried out on a Hitachi liquid chromatograph model 665-II (Hitachi High Technologies, Inc., Tokyo, Japan) equipped with an autosampler (model 655A-40). The stainless steel column (250 × 4.0 mm i.d.) was packed with Inertsil ODS-3v (5μM particle diameter) (GL Sciences, Tokyo, Japan). The column temperature was maintained constant with a Shimadzu column oven CTO-10vp (Shimadzu, Kyoto, Japan). The gradient system consisted of a mixture of acetonitrile and 20 mM KH_2PO_4. The flow rate was 1 mL/min at a column temperature of 30 °C. A Shimadzu photo diode array UV-VIS detector (model SPD-10Avp, Kyoto, Japan) was set from 200 to 700 nm. The tea extract (20 μL) was injected directly into the column. Separate analyses, each in triplicate, were carried out with 3 separate extracts prepared from 3 different tea bags for each sample. The mobile phase used for the separation consisted of acetonitrile (A) and 20 mM KH_2PO_4 (B). The initial composition consisting of 7% A and 93% B was maintained for 7 min. The multiple linear gradients were then programmed for the successful separation the catechins. Separate analyses, each in triplicate, were carried out with 3 separate extracts prepared from 3 different tea bags for each sample.

Statistical analysis

Dunnett's 1-tailed test was used to test for changes from the control (initial value before storage) of individual and total catechin and alkaloid levels in stored teas (SAS 2000).

Table 2—Effect of storage time on the distribution of 7 catechins (CATS) [(−)-epigallocatechin (EGC), (−)-catechin (C), (+)-epicatechin (EC), (−)-epigallocatechin-3-gallate (EGCG), (−)-gallocatechin-3-gallate (GCG), (−)-epicatechin-3-gallate (ECG), (−)-catechin-3-gallate (CG)].[a]

Tea	EGC	C	EC	EGCG	GCG	ECG	CG	Total CATS
Before storage: day 0								
1[b]	ND	ND	12.47 ± 0.45	99.92 ± 1.87	0.65 ± 0.10	44.63 ± 0.93	2.63 ± 0.59	160.30 ± 2.22
2	ND	TR	21.11 ± 0.30	102.79 ± 1.76	0.76 ± 0.06	35.62 ± 0.38	3.34 ± 0.22	163.62 ± 1.84
3	ND	TR	17.38 ± 0.56	88.59 ± 1.70	0.78 ± 0.02	26.89 ± 0.88	2.35 ± 0.16	135.99 ± 2.00
4	TR	0.34 ± 0.06	3.35 ± 0.08	24.19 ± 0.46	0.17 ± 0.02	7.00 ± 0.30	1.49 ± 0.15	36.54 ± 0.58
5	0.02 ± 0	0.09 ± 0.01	13.59 ± 0.27	113.04 ± 3.86	0.98 ± 0.06	39.62 ± 0.30	2.31 ± 0.23	169.65 ± 3.89
6	TR	TR	1.92 ± 0.04	13.33 ± 0.42	1.63 ± 0.09	5.65 ± 0.23	0.53 ± 0.05	23.06 ± 0.49
7	TR	ND	16.49 ± 0.38	78.12 ± 1.22	0.68 ± 0.02	20.74 ± 0.71	2.82 ± 0.11	118.85 ± 1.47
8	ND	ND	7.31 ± 0.22	82.50 ± 1.10	1.23 ± 0.05	50.47 ± 0.43	3.66 ± 0.19	145.17 ± 1.22
Avg	TR	0.05 ± 0.02	11.70 ± 0.33	75.31 ± 1.85	0.86 ± 0.06	28.83 ± 0.58	2.39 ± 0.26	119.15 ± 1.99
1 wk								
1	ND	TR	14.70 ± 0.68	113.14 ± 6.34	0.53 ± 0.08	48.39 ± 1.42	1.71 ± 0.11	178.47 ± 6.53
2	ND	TR	19.04 ± 0.76	90.14 ± 1.77	0.73 ± 0.05	33.46 ± 1.82	2.48 ± 0.12	145.85 ± 2.65
3	ND	TR	15.15 ± 0.72	80.02 ± 2.68	0.34 ± 0.06	23.68 ± 1.60	2.16 ± 0.13	121.35 ± 3.21
4	TR	TR	3.33 ± 0.04	20.30 ± 0.63	0.19 ± 0.02	6.48 ± 0.46	1.61 ± 0.06	31.91 ± 0.78
5	0.06 ± 0.03	0.15 ± 0.03	20.28 ± 0.42	113.2 ± 2.17	1.09 ± 0.02	39.42 ± 0.85	2.69 ± 0.31	176.89 ± 2.39
6	TR	TR	1.98 ± 0.06	12.85 ± 0.40	1.66 ± 0.06	5.46 ± 0.14	3.56 ± 0.17	25.51 ± 0.46
7	ND	TR	14.96 ± 0.42	72.99 ± 1.54	0.84 ± 0.09	19.94 ± 0.88	2.75 ± 0.11	111.48 ± 1.83
8	ND	TR	8.56 ± 0.22	78.65 ± 1.10	1.11 ± 0.05	49.40 ± 1.43	3.21 ± 0.19	140.93 ± 1.83
Avg	0.01 ± 0.01	0.02 ± 0.01	12.25 ± 0.50	72.66 ± 2.72	0.81 ± 0.06	28.28 ± 1.21	2.52 ± 0.17	116.55 ± 3.03
1 mo								
1	TR	0.06 ± 0.02	11.91 ± 0.26	96.49 ± 1.28	0.75 ± 0.10	42.67 ± 0.95	1.64 ± 0.12	153.52 ± 1.62
2	TR	TR	17.64 ± 0.03	88.27 ± 1.36	0.79 ± 0.05	30.28 ± 0.67	2.18 ± 0.22	139.1 ± 61.53
3	TR	TR	14.03 ± 0.47	70.69 ± 2.76	0.41 ± 0.07	21.23 ± 0.81	1.98 ± 0.08	108.34 ± 2.92
4	TR	TR	3.78 ± 0.31	12.40 ± 0.21	0.40 ± 0.01	5.00 ± 0.67	0.17 ± 0.05	21.75 ± 0.77
5	TR	0.13 ± 0.04	10.15 ± 0.27	101.46 ± 2.00	0.67 ± 0.03	34.29 ± 0.57	2.38 ± 0.16	149.08 ± 2.10
6	0.10 ± 0.01	ND	1.94 ± 0.05	11.85 ± 0.67	1.44 ± 0.02	4.80 ± 0.23	2.45 ± 0.28	22.58 ± 0.76
7	TR	TR	13.53 ± 0.87	69.72 ± 0.98	0.47 ± 0.05	19.08 ± 0.30	1.79 ± 0.07	104.59 ± 1.35
8	TR	TR	7.46 ± 0.33	73.59 ± 1.90	0.62 ± 0.04	44.20 ± 0.85	1.77 ± 0.10	127.64 ± 2.11
Avg	0.01 ± 0.00	0.02 ± 0.02	10.06 ± 0.41	**65.56 ± 1.59**[c]	0.69 ± 0.05	25.19 ± 0.68	1.80 ± 0.15	103.33 ± 1.78
2 mo								
1	TR	TR	14.62 ± 0.84	76.07 ± 2.77	0.36 ± 0.02	31.46 ± 0.19	1.47 ± 0.09	123.98 ± 2.90
2	ND	TR	13.95 ± 0.40	71.25 ± 2.37	0.31 ± 0.01	25.50 ± 0.51	1.84 ± 0.09	112.85 ± 2.46
3	ND	TR	11.17 ± 0.35	60.96 ± 2.48	0.30 ± 0.03	18.05 ± 0.39	2.26 ± 0.04	92.74 ± 2.54
4	ND	ND	1.12 ± 0.02	2.35 ± 0.14	0.09 ± 0	3.44 ± 0.09	0.52 ± 0.01	7.52 ± 0.17
5	TR	0.02 ± 0.01	12.91 ± 0.62	88.65 ± 2.77	0.49 ± 0.03	28.45 ± 1.43	1.83 ± 0.04	132.35 ± 3.18
6	TR	ND	1.38 ± 0.05	4.26 ± 0.46	0.11 ± 0	1.62 ± 0.08	0.28 ± 0	7.65 ± 0.47
7	ND	TR	14.32 ± 0.71	55.38 ± 3.10	0.50 ± 0.02	7.72 ± 0.27	2.69 ± 0.04	80.61 ± 3.19
8	TR	0.04 ± 0.01	7.54 ± 0.17	51.70 ± 1.88	0.12 ± 0	37.09 ± 1.02	2.72 ± 0.03	99.21 ± 2.15
Avg	TR	0.01 ± 0.01	9.63 ± 0.49	**51.33 ± 2.25**[c,d]	**0.29 ± 0.02**[c,d]	**19.17 ± 0.67**[c,d]	1.70 ± 0.05	**82.11 ± 2.40**[c]
4 mo								
1	TR	TR	3.49 ± 0.36	73.38 ± 3.90	0.20 ± 0.03	18.92 ± 0.86	1.31 ± 0.03	97.30 ± 4.01
2	TR	TR	8.10 ± 0.43	69.33 ± 2.30	0.46 ± 0.04	8.62 ± 0.53	1.31 ± 0.02	87.82 ± 2.40
3	TR	TR	5.60 ± 0.03	57.54 ± 0.08	0.39 ± 0.01	7.47 ± 0.13	1.58 ± 0.09	72.58 ± 0.18
4	ND	ND	0.17 ± 0.01	2.55 ± 0.01	0.02 ± 0	0.76 ± 0.03	0.14 ± 0.02	3.64 ± 0.04
5	ND	ND	11.94 ± 0.31	71.08 ± 2.61	0.82 ± 0.05	14.88 ± 0.82	1.53 ± 0.04	100.25 ± 2.75
6	ND	ND	1.31 ± 0.12	5.57 ± 0.02	0.14 ± 0.01	0.71 ± 0.08	0.46 ± 0.01	8.19 ± 0.15
7	ND	ND	13.46 ± 0.43	60.22 ± 0.51	0.70 ± 0.04	8.44 ± 0.36	2.16 ± 0.02	84.98 ± 0.76
8	ND	ND	7.26 ± 0.26	56.53 ± 1.85	0.47 ± 0.03	31.42 ± 1.07	0.97 ± 0.05	96.65 ± 2.15
Avg	TR	TR	**6.42 ± 0.29**[c,d]	**49.53 ± 1.97**[c,d]	**0.40 ± 0.03**[c,d]	**11.41 ± 0.61**[c,d]	**1.18 ± 0.04**[c,d]	**68.93 ± 2.08**[c]
6 mo								
1	ND	ND	13.3 ± 0.34	79.03 ± 2.93	0.38 ± 0.03	18.48 ± 2.67	1.89 ± 0.02	113.08 ± 3.98
2	ND	ND	18.96 ± 0.86	73.42 ± 3.09	0.62 ± 0.04	8.56 ± 1.54	1.65 ± 0.05	103.21 ± 3.56
3	ND	ND	15.62 ± 0.17	62.67 ± 2.67	0.54 ± 0.01	14.41 ± 0.15	1.96 ± 0.06	95.20 ± 2.68
4	ND	ND	0.50 ± 0.05	1.61 ± 0.26	0.10 ± 0	1.73 ± 0.12	0.36 ± 0.02	4.30 ± 0.29
5	ND	ND	13.73 ± 0.09	80.49 ± 1.03	0.95 ± 0.05	19.53 ± 0.72	2.01 ± 0.12	116.71 ± 1.27
6	ND	ND	0.14 ± 0.01	2.93 ± 0.22	0.20 ± 0.01	1.31 ± 0.07	0.14 ± 0.02	4.72 ± 0.23
7	ND	ND	15.19 ± 0.29	70.48 ± 1.17	0.77 ± 0.03	13.02 ± 0.44	2.74 ± 0.06	102.20 ± 1.29
8	ND	ND	8.11 ± 0.41	66.25 ± 1.31	0.30 ± 0.04	35.06 ± 2.01	1.81 ± 0.09	111.53 ± 2.44
Avg	ND	ND	**10.69 ± 0.38**[d]	**54.61 ± 1.92**[c,d]	**0.48 ± 0.03**[d]	**14.01 ± 1.34**[c,d]	**1.57 ± 0.06**[d]	**81.37 ± 2.37**[c,d]

[a]Values in mg/g ± SD (n = 3). TR = trace; ND = not detected.
[b]See Table 1 for list of teas.
[c]Indicates that the level of catechin is significantly different ($P < 0.05$) from day 0 using the Dunnett's test.
[d]Indicates that the ratio of catechin to caffeine is significantly different ($P < 0.05$) from day 0 using the Dunnett's test.

Results and Discussion

Catechin content of stored tea leaves

Table 2 shows that all teas evaluated contained no or only trace amounts of EGC and C. In addition, no theaflavins were detected in the green teas listed in Table 1. During storage, the average content of EGCG, the most abundant catechin in the tea varieties, decreased by 28%. ECG, the 2nd most abundant catechin, decreased by 51%. These results indicate that ECG may be more susceptible to degradation than EGCG. Perhaps EGCG is more abundant than ECG in marketplace teas in part because it is more stable. The average overall loss of total catechin concentrations of all 8 teas at the end of the 6 mo of storage was a highly significant 32%.

The plots shown in Figure 2 and 3 visually illustrate the decreases in the values of both individual and total catechin levels of the 8 green tea brands as a function of the 5 storage times. It is not apparent why levels increase from 4 to 6 mo as shown in Figure 2A. One possibility is that volatile ingredients, including water associated with tea ingredients are slowly lost during long-term storage.

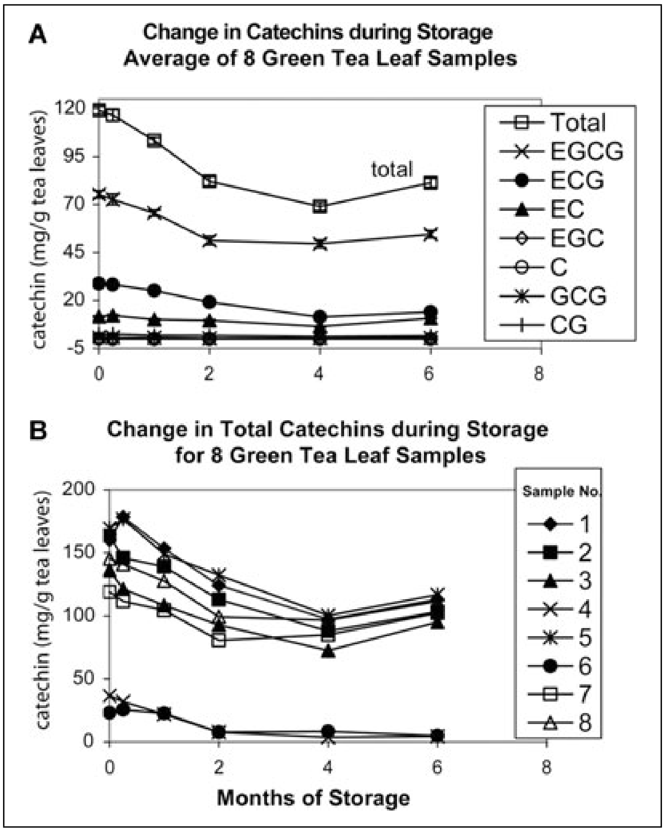

Figure 2—Changes in total and individual catechin concentrations in 8 tea varieties during storage: (A) average individual and total catechin values, (B) decreases over time of total catechins in each tea variety.

Figure 2B shows the variability of total catechins in the 8 tea varieties. All teas show the same continuously decreasing trend over time. Two of the teas contained low initial levels of catechins. This is not surprising since we previously found wide variations in the catechin content of commercial teas sold in the United States (Friedman and others 2005, 2006b). Similar variations are reported for teas sold in Australia (Yao and others 2006).

Because we do not know the history of the teas, it is likely that the teas are of different ages. Variability in catechin content could also be due to genetic variability among the plants from which the leaves were harvested and/or to soil composition, climate, harvesting practices, postharvest storage, sampling, and manufacturing practices (Rusak and others 2008; Sultana and others 2008). Different tea varieties are harvested in different ways and at different times of a year, so that the plants are subjected to different environmental stress conditions.

Conclusions

To our knowledge, this is the 1st report on changes in biologically active green tea catechins during long-term storage of commercial tea leaves in the dry state. The results of the present study for 8 commercial teas of unknown history as well as the results from related studies by other investigators mentioned previously suggest that the consumer needs to be aware about possible adverse consequences of long-term storage of commercial tea brands. Our results show that even in the absence of moisture, quality of teas may degrade with time. Labeling for catechin content and knowledge of storage history may facilitate consumption of the most healthful teas. It is also worth noting that among tea catechins, EGCG, which appears to be susceptible to degradation during storage of the tea leaves, is reported to have the highest activity against bacteria, bacterial toxins, viruses, human cancer cells, and in lipid bilayers of cell membranes (Friedman 2007; Friedman and others 2007; Juneja and others 2007; Sirk and others 2008).

In addition to oxidation products, EGCG can be degraded to GCG or EGCG dimers (Sang and others 2005). We observed no increase in GCG concurrent with EGCG reduction. It needs to be emphasized that we do not know whether the biological/nutritional *in vivo* effects of the transformation/degradation products differ from the corresponding activities of their catechin precursors. Whether new tea ingredients formed during storage, including epimerized and dimerized catechins, will exhibit health-promoting properties after consumption merits further study. The described observations will

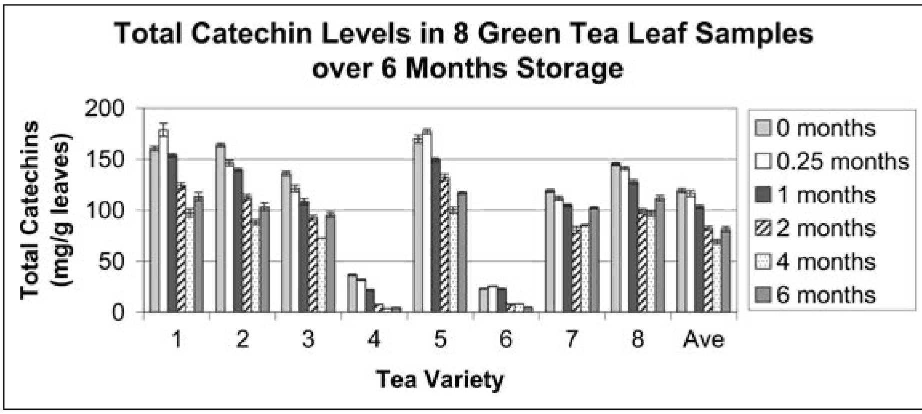

Figure 3—Bar graphs with pooled standard deviations of total catechin levels in green tea brands as function of storage time.

hopefully stimulate additional needed studies on the stabilities of tea catechins in green teas and theaflavins black teas in different brands of tea leaves throughout the harvesting, processing, marketing, storage, and consumption of teas and tea products. Finally, the results of the present study suggest that consumers may benefit from knowing the storage history of teas sold at retail.

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